

Agilent Ref: 10004415-1
United States Application Serial No. 09/740,660

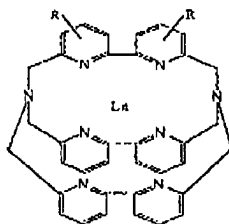
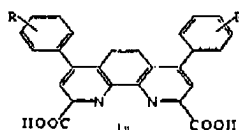
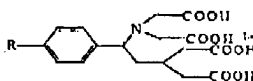
AMENDMENT

In the claims:

1. **(Currently amended)** An encapsulation vesicle, comprising:
 - (a) a synthetic polymeric matrix having a surface;
 - (b) a surface coating on said matrix, wherein said surface coating comprises a fluorescent molecule that is an organo-metallic complex; and
 - (c) a protection layer encapsulating said surface coating, wherein said protection layer permits at least partial transmission of fluorescence emission from said fluorescent molecule upon irradiation of said fluorescent molecule, and wherein said protection layer reduces quenching of said fluorescent molecule **and is capable of excluding small molecules.**
2. **(Previously presented)** An encapsulation vesicle as recited in claim 1, wherein said matrix comprises a sol-gel material.
3. **(Previously presented)** An encapsulation vesicle as recited in claim 1, wherein said matrix comprises silica and synthetic polymer.
4. **(Previously presented)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent molecule is an organo-metallic complex, and wherein the matrix surface is modified with carboxyl groups so that the organo-metallic complex can be covalently attached to the matrix surface.
5. **(Previously presented)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent molecule is an organo-metallic complex, and wherein the matrix surface is modified with amino groups so that the organo-metallic complex can be covalently attached to the matrix surface.

Agilent Ref: 10004415-1
United States Application Serial No. 09/740,660

6. (Canceled)
7. (Canceled)
8. (Canceled)
9. (Canceled)
10. (Previously Presented) An encapsulation vesicle as recited in claim 1, wherein said organo-metallic complex is a ruthenium tris diphenyl phenanthroline complex.
11. (Previously Presented) An encapsulation vesicle as recited in claim 1, wherein said organo-metallic complex has an emission maximum at about 650 nm.
12. (Canceled)
13. (Previously Presented) An encapsulation vesicle as recited in claim 1, wherein said fluorescent molecule is selected from the group consisting of:



Agilent Ref: 10004415-1
United States Application Serial No. 09/740,660

where Ln is selected from the group consisting of Eu, Tb, Sm, and Dy; and R represents H or a functionality capable of covalently linking to the surface of said matrix.

14. **(Previously Presented)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent molecule has a fluorescence lifetime greater than 100 nanoseconds and is susceptible to collisional quenching by oxygen.
15. **(Previously presented)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a material that is translucent to said fluorescence.
16. **(Previously presented)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a material that is transparent to said fluorescence.
17. **(Original)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a sol-gel material.
18. **(Original)** An encapsulation vesicle as recited in claim 2, wherein said protection layer is modified with hydrophilic functionalities selected from the group consisting of hydroxyl, carboxyl and protonated amines.
19. **(Original)** An encapsulation vesicle as recited in claim 2 that was formed by suspension polymerization.
20. **(Previously presented)** An encapsulation vesicle as recited in claim 37, wherein said ligand comprises an acceptor molecule that is capable of absorbing fluorescence that has been emitted from said fluorescent molecule.
21. **(Previously presented)** An encapsulation vesicle as recited in claim 1 further comprising an antibody linked to said protection layer.

Agilent Ref: 10004415-1
United States Application Serial No. 09/740,660

22. **(Canceled)**
23. **(Previously presented)** An encapsulation vesicle as recited in claim 20, wherein an absorption band of said acceptor molecule overlaps with an emission band of said fluorescent molecule.
24. **(Previously presented)** An encapsulation vesicle as recited in claim 20, wherein said acceptor molecule is selected from the group consisting of fluorescein, Cy5 and allophycocyanin.
25. **(Previously presented)** An encapsulation vesicle as recited in claim 37, wherein said ligand is an antibody.
26. **(Canceled)**
27. **(Previously presented)** An encapsulation vesicle as recited in claim 37, wherein said ligand is selected from the group consisting of proteins, DNA, RNA, polypeptides, aptamers and receptor molecules.
28. **(Canceled)**
29. **(Canceled)**
30. **(Canceled)**
31. **(Canceled)**
32. **(Previously presented)** An encapsulation vesicle as recited in claim 21, wherein said vesicle is configured for use in a fluorescence energy transfer immunoassay.

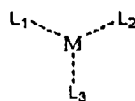
Agilent Ref: 10004415-1
United States Application Serial No. 09/740,660

- 33. **(Previously presented)** An encapsulation vesicle as recited in claim 1 further comprising a polynucleotide linked to said protection layer.
- 34. **(Previously presented)** An encapsulation vesicle as recited in claim 1 further comprising a ligand linked to said protection layer.
- 35. **(Previously presented)** An encapsulation vesicle as recited in claim 1 further comprising an aptamer linked to said protection layer.
- 36. **(Cancelled)**
- 37. **(Currently Amended)** An encapsulation vesicle as recited in claim 1 36 further comprising a ligand attached to said protection layer.
- 38. **(Previously presented)** An encapsulation vesicle as recited in claim 37, wherein said ligand is an antigen.
- 39. **(Previously presented)** An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises silica and synthetic polymer.
- 40. **(Previously presented)** An encapsulation vesicle as recited in claim 20, wherein said acceptor molecule is selected from the group consisting of Fast green and Light green SF yellowish.
- 41. **(Previously presented)** An encapsulation vesicle as recited in claim 20, wherein said acceptor molecule is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, fluorescent infrared-emitting polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines, organo-metallic complexes, and azo dyes.
- 42. **(Previously presented)** An encapsulation vesicle as recited in claim 1 further comprising an acceptor molecule attached to said protection layer,

Agilent Ref: 10004415-1
United States Application Serial No. 09/740,660

wherein said acceptor molecule is capable of absorbing fluorescence that has been emitted from said fluorescent molecule.

43. **(Previously presented)** An encapsulation vesicle as recited in claim 42, wherein said acceptor molecule is selected from the group consisting of Fast green and Light green SF yellowish.
44. **(Previously presented)** An encapsulation vesicle as recited in claim 42, wherein said acceptor molecule is selected from the group consisting of fluorescein, Cy5 and allophycocyanin.
45. **(Previously presented)** An encapsulation vesicle as recited in claim 42, wherein said acceptor molecule is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, fluorescent infrared-emitting polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines, organo-metallic complexes, and azo dyes.
46. **(Previously presented)** An encapsulation vesicle as recited in claim 42, wherein an absorption band of said acceptor molecule overlaps with an emission band of said fluorescent molecule.
47. **(Previously presented)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent molecule is susceptible to collisional quenching by oxygen and said protection layer reduces the diffusion of oxygen into said surface coating.
48. **(Currently Amended)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent molecule is:

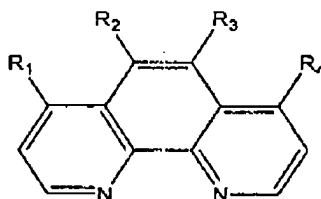


Agilent Ref: 10004415-1
United States Application Serial No. 09/740,660

where M is selected from the group consisting of Ru, Os and Re; and
L₁-L₃ are each independently selected from the group consisting of:

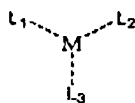


and



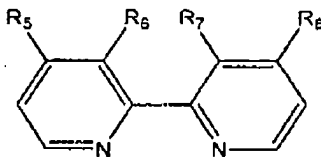
where R₁-R₈ are each independently selected from the group consisting of H, alkyl and aryl.

49. **(Previously Presented)** An encapsulation vesicle as recited in claim 1, wherein said fluorescent molecule is:



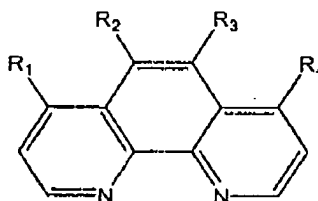
where M is Os;

L₁ is:

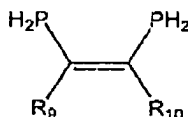


Agilent Ref: 10004415-1
 United States Application Serial No. 09/740,660

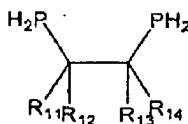
; or



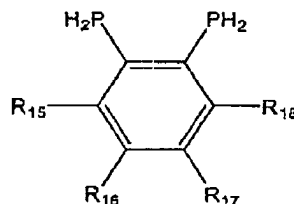
; and L_2 and L_3 are independently selected from the group consisting of:



;



; and



where R_1 - R_{18} are each independently selected from the group consisting of H, alkyl, and aryl.

50. (New) An encapsulation vesicle as recited in claim 1, wherein said protection layer comprises a sol-gel.